

# Genetic Characterization and High Efficiency Photosynthesis of an Aurea Mutant of Tobacco

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## ABSTRACT

A new tobacco (*Nicotiana tabacum*) aurea mutant was isolated from the progeny of a selfed variegated tobacco plant. The new mutant is termed Su/su var. Aurea. If the mutant is selfed, the seeds obtained give rise to four types of plants: green seedlings which correspond to the wild type; yellow-green seedlings which correspond to the earlier described Su/su; yellow seedlings which correspond to the new tobacco aurea mutant Su/su var. Aurea; and white lethal seedlings. The frequency ratio of the four phenotypes is 1:1:1:1. It appears that the mutation is due to two independent nuclear factors, su and aur, both of which have to be present in a heterozygous condition, Su/su Aur/aur, to give rise to the new aurea phenotype. The aurea mutant Su/su var. Aurea has a reduced photosynthetic unit size which is approximately one-eighth of the wild type. Despite its chlorophyll deficiency, the plant grows well and exhibits maximal photosynthetic rates on a chlorophyll basis which are at least seven times higher than those of the green wild type provided the temperature and the light intensities are high enough. In contrast to the earlier described Su/su, the new mutant does not exhibit more photorespiration than the wild type. It appears that the factor aur causes either repression of photorespiration or an increase in the number of functioning photosynthetic units.

The current goal of plant breeding must be the production of plants which under specified environmental conditions give maximal crop yields. Photosynthesis is the process by which solar energy is converted into the chemical energy of organic plant materials. It is obvious that the prerequisite for optimal growth is an optimal rate of photosynthesis provided that properties which counteract high rates of apparent photosynthesis such as photorespiration (22), a low photosynthetic water use efficiency (1), and others are eliminated. In most green plants, the dependence of the maximal rate of photosynthesis on the light intensity clearly shows that at high light intensities the light-utilizing capacity is low. At high light intensities, most of the light energy absorbed by the chloroplast pigments is wasted because most green plants contain a great excess of Chl in relation to their photosynthetic capacity. The accepted explanation is that the activities of dark "enzymes" are not sufficient to make use of the flux of primary photochemical products (6). This excess of light-catching capacity over the light-utilizing capacity in normal green plants is due to the structure of the photosynthetic apparatus in which several hundred Chl molecules feed the absorbed light energy into an energy trap or a reaction center. This insight that only a fraction of the Chl present in a plant directly takes part in photochemistry whereas the major part simply plays the role of a

light antenna, is the basic principle of the photosynthetic unit first advanced by Gaffron and Wohl in 1936 (8). The experimental basis for this concept was provided by the classic experiments of Emerson and Arnold (6, 7). It appeared later that many green plants have similar maximal rates of photosynthesis on the basis of Chl (15), which means that they have approximately the same ratio of effective pigments to dark enzymes and in turn may mean that the sizes of their light antennae are similar. A dominant aurea mutant from tobacco, on the other hand, and some other Chl-deficient tobacco mutants reach maximal rates of photosynthesis which are much higher than in normal plants on a Chl basis and therefore permit a much better photochemical utilization of high light intensities (15, 16). The photosynthetic units of these plants are smaller than in green plants (18).

In the present paper we report on the properties of a peculiar aurea mutant from tobacco which is distinctly more Chl-deficient than the tobacco mutant Su/su (15) described earlier, or than the barley mutant found by Highkin (10). Yet it grows under certain conditions with a good growth rate and reaches the flowering state within the time usual for most tobacco varieties.

## MATERIALS AND METHODS

**Plant Material.** The wild type tobacco described in this paper is the Connecticut cigar variety *Nicotiana tabacum* cv. John William's Broadleaf (JWB).<sup>2</sup> Su/su is the dominant aurea mutant of tobacco first described by Burk and Menser (3). All tobacco varieties described here were grown in the greenhouse and received continuous artificial illumination during the winter months from October to April.

**Chloroplast Preparations.** Chloroplasts were prepared according to Homann and Schmid (11).

**Manometric Analyses.** Oxygen gas exchange rates were measured in a Warburg apparatus as described earlier (15) using the conventional carbonate-bicarbonate buffer system. Leaf sections 2 cm<sup>2</sup> were placed on a stainless steel spiral in the Warburg flask and illuminated from below.

**Infrared CO<sub>2</sub> Analyses.** The CO<sub>2</sub> exchange rates and CO<sub>2</sub> compensation points were determined in a closed system with an IR analyzer (Beckman model 865 IR analyzer or Hartmann and Braun URAS-2). The system was equipped with a Hartmann and Braun membrane pump and a gas flow meter. By means of four-way stopcocks, the system was connected with a humidifying device with a CO<sub>2</sub> absorber or an O<sub>2</sub> absorber (Cu-CuO combustion tube) and to the various gas mixtures with which the system could be flushed. The volume of the entire system was 765 cm<sup>3</sup>. The gas flow rate was 1,000 cm<sup>3</sup>/min. The tobacco leaflet stood in water in a flat thermostated Plexiglas chamber (8

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<sup>2</sup> Abbreviations: JWB: *N. tabacum* var. John William's Broadleaf; PMS: phenazine methosulfate.

$\times 5 \times 0.8 \text{ cm}^3$ ) and was illuminated from the side with a projector lamp (Leitz Prado Universal Projektor). The rates of  $\text{CO}_2$  uptake or evolution described are averages of a great number of experiments and are steady rates under the conditions indicated. About 10 to 15 min were necessary until the rates of photosynthesis reached their constant level which corresponds to the indications given earlier by Zelitch and Day (23).

**Light Intensity.** The incident light intensity was measured at the place of the leaf sample with an ISCO spectroradiometer (Lincoln, Nebr.) essentially as described earlier (17).

**Partial Reactions of Photosynthesis and Photosynthetic Unit Sizes** were measured as described earlier by Homann and Schmid (11) or Schmid *et al.* (20) and Schmid and Gaffron (18).

## RESULTS

**Origin of the Tobacco Aurea Mutant Su/su var. Aurea.** In a seed lot derived from a selfed Su/su mutant which was first described by Burk and Menser (3) we observed a peculiar variegated plant (Fig. 1). The variegation consisted of yellow-green leaf areas which were already Chl-deficient and exhibited physiological properties such as maximal rates of photosynthesis, very similar to that described earlier for Su/su. The other leaf areas were intensely yellow, much more Chl-deficient, and exhibited maximal rates of photosynthesis superior to the previously described Su/su.

**Genetic Characterization of the Tobacco Mutant Su/su var. Aurea.** This variegated plant was selfed and yielded progeny with four types of plants: green wild type plants; yellow green

plants which cannot be distinguished from Su/su; yellow plants of a hitherto undescribed kind; and lethal plants. The yellow plants were found to be viable (Fig. 2). Selfing of these yellow plants yielded a seed lot from which four types of seedlings with the frequency ratio 1:1:1:1 emerged (Table II). The Chl content of the yellow plants/leaf area in comparison to the green plant and the yellow-green Su/su is shown in Table I. Selfing of these plants or crossings with JWB and Su/su gave seed lots with the type distribution shown in Table II. The color differences seen in Figure 2 are already clearly recognizable in the cotyledonary stage. It appears that the characteristics of yellow-green and yellow are due to two unlinked gene pairs designated as Su/su and Aur/aur (Table II). The designation Su/su was originally introduced by Burk and Menser for the condition of the dominant aurea mutant which they described in 1964 (3) and from which the mutant Su/su var. Aurea described in this paper was derived.

It appears that the four phenotypes described have the following gene constitution: *green plants* carry su/su Aur/Aur or su/su Aur/aur. The condition su/su aur/aur cannot occur because the pollen with su aur is lethal (Table II); *yellow-green plants* carry exclusively Su/su Aur/Aur; *yellow plants* have the condition Su/su Aur/aur, that is, both genetic factors are present in a heterozygous condition. This is the new aurea mutant which we term Su/su var. Aur. *Lethal plants* are those with the combination Su/Su Aur/Aur or Su/Su Aur/aur. Su/Su aur/aur cannot occur as the pollen with Su/aur also appears to be lethal (Table II). With these gene combinations all of the distributions shown in Table II can be fully explained. In this context it must only be borne in



FIG. 1. Variegated tobacco plant isolated from a seed lot of selfed *N. tabacum* var. Su/su.





FIG. 2. Tobacco plants which were obtained from the seed lot of the selfed variegated plant shown in Figure 1. Besides the three plant types shown, the seed lot also yielded white lethal cotyledons which died shortly after germination. The plants shown are 6 to 8 weeks old.

Table I Pigment Content in Leaves of the Tobacco Mutant *N. tabacum* Su/su var. Aurea

Tobacco Variety	Chlorophyll Content per Leaf Area				
	Chl <sub>a</sub>	Chl <sub>b</sub>	total Chl	$\frac{\text{Chl}_a}{\text{Chl}_b}$	$\frac{\text{Total Carotenoid}}{\text{Total Chlorophyll}}$
	$\mu\text{g}/\text{cm}^2$			Ratio	
young leaves					
John William's Broadleaf	17.34 $\pm$ 2.18	7.65 $\pm$ 0.67	24.99 $\pm$ 7.08	2.267	0.058 $\pm$ 0.001
Su/su	7.57 $\pm$ 0.49	2.59 $\pm$ 0.42	10.16 $\pm$ 0.9	2.915	0.103 $\pm$ 0.014
Su/su var. Aurea	2.49 $\pm$ 0.45	0.87 $\pm$ 0.43	3.35 $\pm$ 0.9	2.86	0.219 $\pm$ 0.013
fully expanded leaves					
John William's Broadleaf	32.6 $\pm$ 3.4	10.4 $\pm$ 0.6	45 $\pm$ 0.1	3.13	0.056 $\pm$ 0.001
Su/su	6.85 $\pm$ 1.75	1.16 $\pm$ 0.34	8 $\pm$ 2	5.9	0.079 $\pm$ 0.003
Su/su var. Aurea	4.35 $\pm$ 0.35	0.74 $\pm$ 0.16	5.08 $\pm$ 0.32	5.88	0.117 $\pm$ 0.006

Table II Seedling Ratios in Seeds Derived from Crossings between Green, Yellow-green and Yellow (Aurea) Tobacco Plants

No. Crossing		normal green	yellow-green	yellow (Aurea)	white (lethal)
1	green <sup>1</sup> x green <sup>1</sup>	all	0	0	0
2	yellow-green x yellow-green	168	325	0	160
3	yellow (Aurea) x yellow (Aurea)	585	573	578	554
4	green <sup>1</sup> x yellow-green	1330	1293	0	0
5	green <sup>1</sup> x yellow (Aurea)	754	729	0	0
6	yellow-green x green <sup>1</sup>	808	779	0	0
7	yellow (Aurea) x green <sup>1</sup>	373	202	195	0
8	yellow-green x yellow (Aurea)	65	121	0	65
9	yellow (Aurea) x yellow-green	338	290	288	306

<sup>1</sup> The source of green plants is JWB

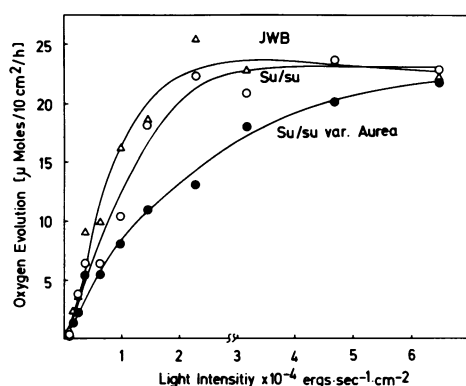


FIG. 3. Dependence of photosynthesis on the light intensity measured as O<sub>2</sub> evolution/leaf area in the three types of tobacco. Conditions: 0.45% CO<sub>2</sub> in air at 30 C in red light transmitted through a red Plexiglas filter 580 nm < λ < 700 nm. Light intensities higher than 30,000 ergs·sec<sup>-1</sup>·cm<sup>-2</sup> were obtained with white light 380 nm < λ < 700 nm. Averages of at least four determinations. JWB: green wild type; Su/su: yellow-green; Su/su var. Aurea: yellow plants.

mind that pollen with the condition *aur*, regardless whether present as Su/aur or su/aur, is lethal.

**Maximal Rates of Photosynthesis and Rates of Photorespiration in the Tobacco Mutant Su/su var. Aurea.** The comparison of Chl content and rates of photosynthesis between the wild type and the mutant on the basis of leaf area is difficult to assess because both values depend on the leaf thickness, which is different in both types of plants. The leaf thickness in turn depends on the environmental conditions such as light intensity, day length, and temperature to which both types of plants react in an entirely different manner. For the early stage of growth, 6 to 8 weeks after germination, the Chl and carotenoid content/leaf area is seen in Table I. The rate of photosynthesis at 30 C in a saturating CO<sub>2</sub> atmosphere of 0.45% is shown in Figure 3. It is clear that the mutant reaches the rate of the green control, however, only at higher light intensities. The situation is similar to that of the earlier described Su/su (15).

However, the photosynthetic capacity of the mutant is immediately recognized if rates of photosynthesis are calculated on a Chl basis. At 20 C the new mutant has a rate of photosynthesis which is at least five times higher than the green control (Fig. 4). Saturation of photosynthesis is reached at three to five times higher light intensities. At 30 C the difference in photosynthetic capacity in comparison to the previously described Su/su and the wild type further increases on a Chl basis (Fig. 5). The maximal rate in the new mutant is at least seven times higher than in the green control without having reached light saturation at an intensity of 60,000 erg·sec<sup>-1</sup>·cm<sup>-2</sup>. It seems that the mutant

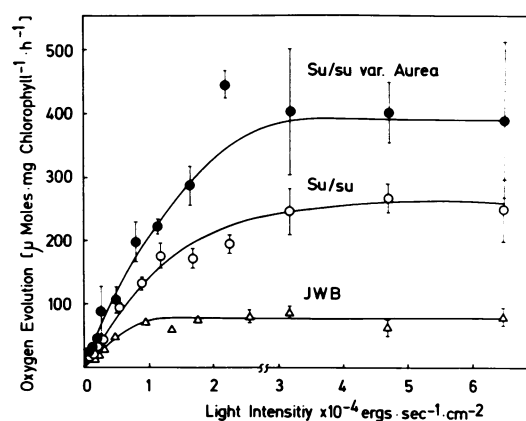


FIG. 4. Dependence of photosynthesis on the light intensity on a Chl basis in the three types of tobacco. Conditions: 0.45% CO<sub>2</sub> in air at 20 C in red light 580 nm < λ < 700 nm. White light was used to obtain light intensities above 30,000 ergs·sec<sup>-1</sup>·cm<sup>-2</sup>. Values with standard deviations.

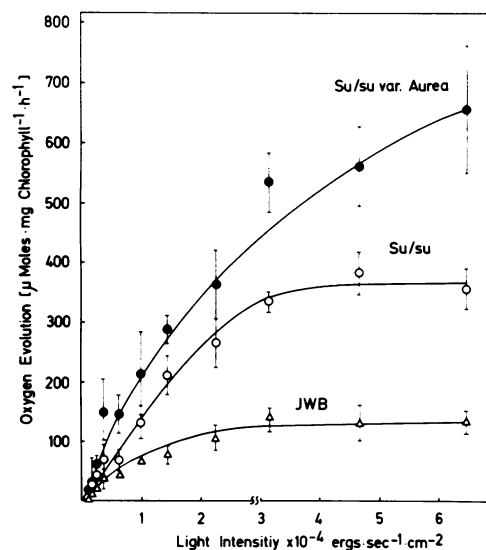


FIG. 5. Dependence of photosynthesis on the light intensity on a Chl basis at 30 C. Values with standard deviations. Other conditions as in Figure 3.

requires high light intensities for optimal growth and temperatures above 30 C. Since the results of Figures 3 to 5 are obtained in red light, this means that the increased carotenoid to Chl ratio seen in Table I is of no special advantage to the plant.

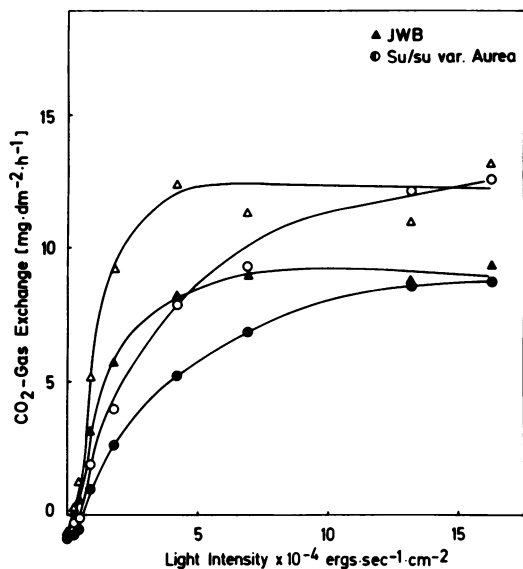


FIG. 6. Photosynthesis/leaf area in dependence on the light intensity at 300  $\mu\text{l/l}$   $\text{CO}_2$  in air (21%  $\text{O}_2$ ,  $\bullet$ ,  $\blacktriangle$ ) and 300  $\mu\text{l}$   $\text{CO}_2$  in nitrogen (1.7%  $\text{O}_2$ ,  $\circ$ ,  $\triangle$ ). Averages of at least three determinations. Temp. 25 C. ( $\triangle$ ,  $\blacktriangle$ ) JWB; ( $\circ$ ,  $\bullet$ ) Su/su var. Aurea.

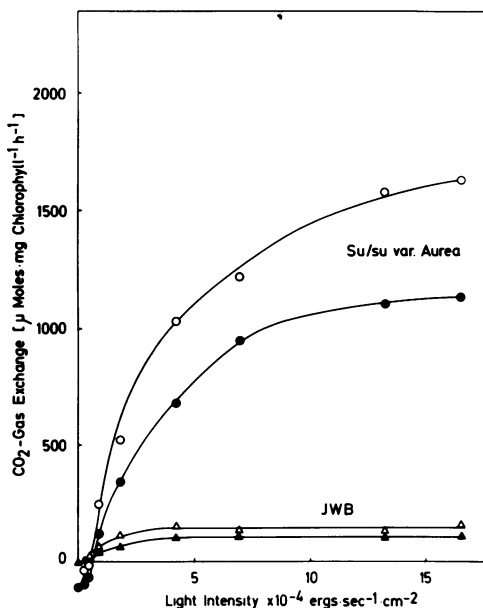


FIG. 7. Dependence of photosynthesis/Chl on the light intensity at 300  $\mu\text{l/l}$   $\text{CO}_2$ . The curve shows the effect of anaerobic (1.7%  $\text{O}_2$ ,  $\circ$ ,  $\triangle$ ) and aerobic (21%  $\text{O}_2$ ,  $\bullet$ ,  $\blacktriangle$ ) conditions on the photosynthetic rates in the wild type tobacco (JWB) and the tobacco aurea mutant Su/su var. Aurea. Otherwise conditions as in Figure 6.

At low  $\text{CO}_2$  partial pressure the maximal rates of photosynthesis are also comparable to those of the wild type (Fig. 6). Since Zelitch and Day (23) had reported earlier that the Su/su mutant exhibited unusually high rates of photorespiration, we suspected that the mutant Su/su var. Aurea might also show this disadvantageous property in a pronounced form. Because photorespiration is suppressed by low partial pressures of  $\text{O}_2$  (5), we compared rates of photosynthesis in 0.03%  $\text{CO}_2$  at normal  $\text{O}_2$  pressure (21%) and at low  $\text{O}_2$  pressure (1.7%). In the early stage of growth (6–8 weeks after germination), we found no significant difference in comparison to the wild type (Fig. 6). This means that the mutant Su/su var. Aurea in the early stage of growth does not exhibit significantly more photorespiration than

the wild type. In both cases, low  $\text{O}_2$  pressure increases the maximal rates of photosynthesis between 20 and 30% (Fig. 6). If the same data are expressed on a Chl basis (Fig. 7), it is seen (due to the amplifying effect with Su/su var. Aurea) that the dependence of photorespiration (*i.e.* the difference between the two curves) on the light intensity starts with a lag until the region of approximately 1,500  $\text{ergs} \cdot \text{sec}^{-1} \cdot \text{cm}^{-2}$  and then increases with the light intensity until eventually a light saturation is reached (seen with JWB). The result is a sigmoidal curve shape which hints at a cooperative effect in photorespiration. This interpretation is correct if the difference between rates of photosynthesis at high and low  $\text{O}_2$  partial pressures really measures photorespiration, which is questioned somewhat by D'Aoust and Calvin (4).

As to Figure 7, it should be noted that all light intensity curves are more or less sigmoidal as shown by Wang and Myers (21). By principle, a plant with a small photosynthetic unit such as Su/su var. Aurea will show this effect in a more pronounced way than a plant with a normal unit size. In essence the sigmoidal shape of a light intensity curve means a decreased quantum yield which according to the literature is explained by the S-state inactivation of the  $\text{O}_2$ -evolving mechanism (12, 13, 21).

The comparison of the  $\text{CO}_2$  compensation points in the two plants shows that the mutant can fix  $\text{CO}_2$  down to a concentration of around 50  $\mu\text{l/l}$   $\text{CO}_2$  in the ambient atmosphere which is a typical final value for  $\text{C}_3$  plants. However, the light dependence is different from the green control showing that at a lower than saturating light intensity the  $\text{CO}_2$  requirement in the mutant plant is much higher, which certainly is a disadvantage to the plant (Fig. 8). In this context it should be noted that Heichel has recently observed that the Su/su tobacco mutant was more sensitive to  $\text{O}_2$  than the wild type (9).

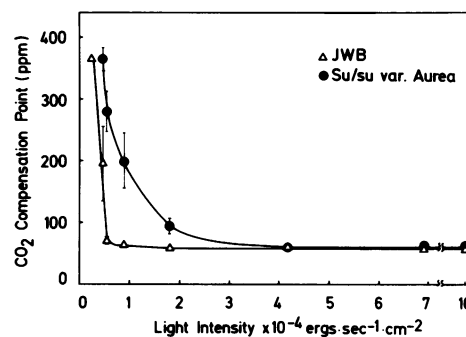


FIG. 8. Influence of the light intensity on the  $\text{CO}_2$  compensation point in wild type tobacco and the tobacco aurea mutant *N. tabacum* Su/su var. Aurea. Values are the averages of at least three independent determinations. Standard deviations are indicated.

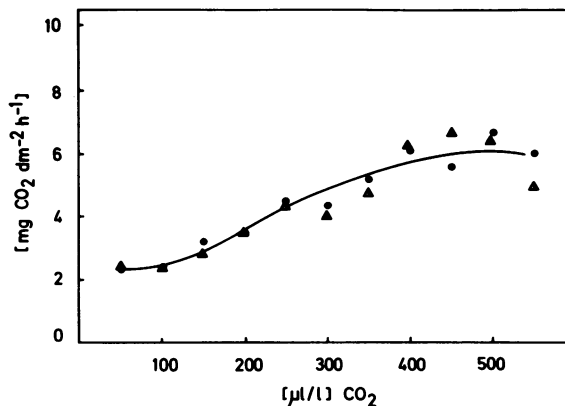


FIG. 9. Dependence of photorespiration on the  $\text{CO}_2$  concentration in the ambient atmosphere. Photorespiration was measured as the difference of photosynthetic rates in low (1.7%)  $\text{O}_2$  partial pressure and high (21%)  $\text{O}_2$  partial pressure. 165,000  $\text{ergs} \cdot \text{sec}^{-1} \cdot \text{cm}^{-2}$  of red light 580 nm  $< \lambda < 700$  at 25 C. ( $\triangle$ ) JWB; ( $\bullet$ ) Su/su var. Aurea.

Table III Photosynthetic Unit Sizes in Intact Leaves and Activities of Partial Reactions of Photosynthesis in Isolated Chloroplasts of Wild Type Tobacco, the Dominant Tobacco Aurea Mutant Su/su and the New Tobacco Aurea Mutant Su/su var. Aurea

	wild type	Su/su	Su/su var. Aurea
Photosynthetic Unit Sizes [chlorophyll molecules/molecule CO <sub>2</sub> -fixed/flash]	2400 ± 700	600 ± 180	300 ± 100
H <sub>2</sub> O Ferricyanide [μMol ferricyanide reduced /mg chlorophyll/h]	800 - 1400 <sup>+</sup>	2100 - 3400 <sup>+</sup>	2000 - 7000 <sup>+</sup>
Photophosphorylation Reactions [μMol <sup>32</sup> P esterified/mg chlorophyll/h]			
H <sub>2</sub> O → Ferricyanide	65 ± 10	335 ± 75	455 ± 65
H <sub>2</sub> O → Methylviologen	30 ± 5	-	152 ± 34
PMS	500 ± 50	1100 ± 100	2500 ± 300

<sup>+</sup>The rates of the ferricyanide-Hill reaction are uncoupled rates and decrease quickly with time after the chloroplast preparation. Temp. 20 C for all reactions. Saturating light intensities.

The dependence of photorespiration on the CO<sub>2</sub> concentration in the ambient atmosphere also shows that there is no difference in the photorespiratory activity in the wild type and the aurea mutant (Fig. 9). Hence, in contrast to the earlier described aurea mutant Su/su, the new mutant Su/su var. Aurea has the same photorespiratory activity as the wild type.

**Photosynthetic Unit Sizes and Partial Reactions of Photosynthesis in the Tobacco Aurea Mutant Su/su var. Aurea.** Up to now it appears that in the new tobacco mutant only the size of the light antenna has been decreased. The photosynthetic unit size measured in intact leaves with xenon flashes as the number of Chl molecules involved in the fixation of 1 molecule CO<sub>2</sub> (Table III) or in the evolution of 1 molecule O<sub>2</sub> (data not shown; Renger and Schmid in preparation) comes out smaller than the wild type. The reduced unit size corresponds roughly to the reduced Chl content. Electron transport reactions and photophosphorylation reactions in isolated chloroplasts appear to yield rates which are distinctly superior to those of the earlier described Su/su and are certainly better than with chloroplasts from the wild type (Table III). The chloroplast structure resembles the structure described earlier for Su/su (19). The lamellar system of the mutant is composed of extended intergrana regions with occasional doublings and overlappings of thylakoids. However, the chloroplast structure is distinctly different from that of chloroplasts from the yellow leaf patches of the variegated tobacco mutant NC95 which was described by Homann and Schmid (11). In such chloroplasts isolated single thylakoids were distributed in the stroma with no detectable thylakoid overlappings. These chloroplasts exhibited only photosystem I activity (11).

## DISCUSSION

The principal characteristic of the tobacco aurea mutant Su/su var. Aurea is the smaller photosynthetic unit size in comparison to the green control, a fact which permits a more efficient light energy conversion at high light intensities (Table III). In contrast to the earlier described Su/su (3, 11, 15, 23), it appears that this mutant does not exhibit a higher rate of photorespiration than the wild type. As Zelitch and Day (23) showed earlier that the wild type JWB exhibited an especially low photorespiration, it would appear that Su/su var. Aurea also has an especially low photorespiration. This might be an explanation for why this mutant grows so well despite its Chl deficiency. To our knowledge there is no report in the literature on a plant with a Chl deficiency comparable to that of Su/su var. Aurea which grows nearly as well as this mutant. The most frequent situation in Chl-deficient mutants is apparently a reduction of the unit size

together with a reduction of the number of functioning photosynthetic units. This explains the usual poor growth rates even at high light intensities of most of the Chl mutants which we have investigated in the past 10 years. An exception to this was the dominant tobacco aurea mutant Su/su (3, 15). However, later it became evident that this plant in certain growth stages exhibited under natural environmental conditions (300 μl/l CO<sub>2</sub>) unusually high rates of photorespiration (14, 23). From our results with Su/su var. Aurea it appears very probable that despite the reduction of antenna Chl an increase in the functioning numbers of photosynthetic units has occurred or at least no significant decrease. From Figures 4, 5, and 7 and Table III it is quite obvious that a plant with nearly normal Chl content but the better functioning photosynthetic apparatus of Su/su var. Aurea would really improve on the known efficiency of solar energy conversion in higher plants. In order to achieve this, one might think of a recombination of cell organs (2) or their mixing (2). However, as the property is controlled by the nucleus, fusions between chloroplast-free cells and Su/su var. Aurea chloroplasts or between green and aurea type chloroplasts will give no useful result.

We have shown that the mutation is due to the introduction of the two factors su and aur both of which have to be present in a heterozygous condition Su/su Aur/aur to give rise to the mutant plants. The question arises as to what new property is introduced by the factor aur. If the factor aur causes suppression of photorespiration or increase in unit numbers, the green type plants which emerge from the crossing of Su/su var. Aurea × JWB (No. 7 in Table II) will be of interest. These plants obviously differ with respect to their gene constitution. Half of the emerging plants will have the condition su/su Aur/aur, the other half the condition su/su Aur/aur which might lead to a difference with respect to the photorespiratory or photosynthetic activity. Moreover, the production of haploid (su aur) plants from other cultures of Su/su var. Aurea or from the green type su/su Aur/aur of crossing No. 7 should provide new insights. The condition Su/Su aur/aur will be of special interest.

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